Evidence of Youthful Aging: Chronic Stress and the Association with DNA Damage

Jason Fly and M. Catherine DeSoto, Ph.D.

University of Northern Iowa

Abstract

Persistent elevated basal levels of the hormone cortisol are an indication of chronic stress. Maladaptive response to stress, or chronic psychological stress, is thought to play a crucial role in the biological mechanisms involved in mental disorders, disease, and accelerated aging. In 2011, a critical connection was reported between elevated cortisol and the oxidative damage to DNA associated with aging and disease in a study of elderly participants (ages 63-83) via 24-hour urinary samples (Joergensen, et al., 2011). This connection, if verified, has implications for how stress may accelerate the aging process and the onset of cancer, diabetes, and other diseases. The possible relationship between psychological stress and the cellular damage that underlies aging and disease is explored here, replicating the prior study with a sample of 49 young adults (ages 18-26) via direct salivary assay. Results show a significant association was also found, suggesting a link between elevated cortisol and DNA damage at earlier ages. Potential clinical impacts and suggestions for further research are discussed.

Keywords: chronic stress; cortisol; DNA damage

Chronic psychological stress, as measured by the elevation of the stress hormone cortisol, is thought to play a crucial role in the biological mechanisms involved in disease and accelerated aging. Joergensen, et. al., (2011) found a crucial association between cortisol and the oxidative damage to DNA, in a study of elderly participants (age 63-83). This relationship has implications for how stress may fundamentally change underlying cellular processes, and alter the function of DNA. The study of epigenetics is relatively new, and importantly, the possible epigenetic connection between psychological stress and the cellular damage that underlies future aging and disease has not yet been explored with younger persons.

Stress Response

The human stress response system consists of the hypothalamus region of the brain, the pituitary gland, and the adrenal cortex (HPA axis). When a stressful event occurs, this axis is stimulated to produce stress hormones to aid in physiological and neurological functioning during and after the stressful event (Kozlov & Kozlova, 2014). Stress response has been associated with an individual's ability to manage or diminish the negative effects of threat or anxiety. However cortisol, the main hormone associated with the stress response system, functions as an adaptive substance designed for optimal performance when challenged (Aschbacler, et al., 2013).

Basal levels of cortisol are released into the bloodstream at varying levels consistently to maintain cellular homeostasis (Kozlov, 2014). Under normal conditions, cortisol levels are highest in the morning soon after awakening, and steadily decline throughout the day reaching the lowest levels in the early evening as the body prepares for sleep (Fig. 1). This pattern reflects cortisol's preparatory role both in the expenditure and conservation of energy. Cortisol is involved in cognitive capacities such as memory and learning, and metabolic functioning

(Aschbacher, et.al., 2013). Beneficial elevations of cortisol occur during physical exercise or engaging in complex tasks (Hill, Battaglini, Viru, & Hackney, 2008). Despite the crucial functions of this neuroendocrine molecule, the terms “stress” and “cortisol” have negative connotations.

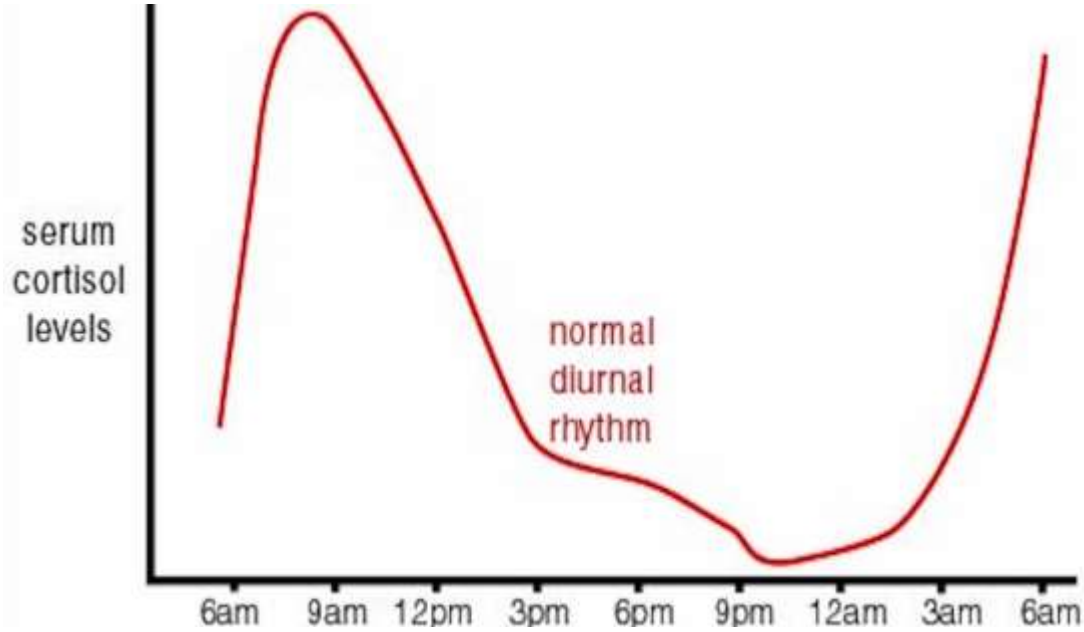

Fig. 1

Normal basal cortisol excretion pattern as a function of time of day.

Chronic Stress

Maladaptive response to stress, or chronic psychological stress, has been shown to have detrimental effects to health (Flint, Baum, Chambers, & Jenkins, 2007). Chronic psychological stress can be defined as a persistent perception of a challenge or threat, although no specific stressful stimulus is present (Wang, et al., 2007). When exposed to consistently high levels of cortisol, the adaptive benefits of enhanced performance and repair begin to reverse. Persistent increased levels of basal cortisol are associated with mental disorders, such as anxiety and

depression, and decreased learning and memory capacities. In animal models, chronic stress induced in adolescent mice resulted in cognitive dysfunction in older ages, suggesting a developmental long-term link of abnormally high cortisol and cognitive decline (Sterlemann, et al., 2010).

In a study to test the hypothesis that moderate levels of stress are actually beneficial to health, Aschbacher, et al. found that stress reactivity might reflect an inverted U-shaped or J-shaped curve where optimum performance is at the apex, representing a moderate stress condition. The authors also examined whether a chronic stress condition would predispose participants to exhibit oxidative cell damage when exposed to an acute stressor. Participants were recruited from a post-menopausal pool of women of similar ages, with one group being caregivers of spouses with dementia (chronic stress condition) and the other group with healthy spouses (control group). The chronic stress group showed higher levels of a DNA biomarker for oxidative cell damage when exposed to the stressor compared to control. Interestingly, control participants who rated themselves as low in perceived levels of stress in a pre-experiment scale, had expected low levels of DNA damage, but slower returns to baseline levels of cortisol when compared to those rated as moderate in perceived stress.

Short-term examinations of acute stress effects on repair mechanisms however, do not yield the same patterns of DNA damage (Forlenza, Latimer, & Baum, 2000). In a study of graduate students taking blood samples to measure for DNA repair levels during both low-stress and high-stress periods, DNA repair increased during high levels of stress as a buffer to damage (Forlenza, et al., 2000). This illustrates the distinction between methods of measurement and differences in effects of acute versus chronic stress conditions. This may also demonstrate a biological explanation for the many benefits associated with regular exercise, including

preserving memory and improving immune function that periodic elevations of cortisol as opposed to chronic elevation levels produce (Hill, et al., 2008).

Chronic stress may be, from a biological point of view, an optimization of the stress response system to respond to anticipation of stressors. In effect, the body tries to prepare one for the more likely chance that one will encounter stressful events, with the unfortunate side effect of accelerating aging and disease (Aschbacher, et al., 2013). One theory of the role of cortisol in the aging process is the interference of a cell's inherent repair system to neutralize the oxidative molecules naturally produced during metabolic processes. Over time, mitochondrial structure is altered and the oxidation of cells exceeds the repair mechanisms causing cells to die or mutate (Flint, et al., 2007). Generally speaking, excess cortisol over time as represented by chronic psychological stress, turn once thriving cells into inefficient and dying ones.

Aging and DNA Damage

A physiological response to elevated cortisol in aging results in reduced: learning ability, memory formation, T-cell formation, muscle mass, and bone mass (Kozlov & Kozlova, 2014). Elderly men generally have a flatter diurnal cortisol curve, showing signs of accelerated aging relative to women which may be due to higher cortisol reactivity to acute stress or cumulative stress effects over the lifespan (Wang, et al., 2007). The major theories to explain the aging process include: Evolutionary theory, in which mutations that are not harmful at younger ages deteriorate in late life to preserve resources for the young; Molecular theory, in which inefficiencies accumulate due to changes in gene expression; Neuroendocrine theory, in which disruptions in homeostasis results in altered physiology; Free-radical theory, in which cells are damaged by an inability to remove oxidative by-products; and Telomere theory, in which cells lose their ability to replicate (Hasan, Rahman, Arif, & Sobhani, 2011). To date, however, the

biological mechanisms underlying the role of excess cortisol in aging and disease has yet to be elucidated.

Concentrating on the effects of chronic stress alone as opposed to acute stress warrant more examination, especially considering the possible heritability of stress reactivity (Steptoe, et al., 2009) and gender differences in response to stress (Wang, et al., 2007). With advances in assays and more non-invasive techniques, the question of how to best measure chronic levels of cortisol are becoming clearer. In 2011, a vital connection was reported between elevated cortisol and the oxidative damage to DNA in a study of elderly participants (ages 63-83) via 24-hour urinary samples (Joergensen, et al., 2011). This connection, if verified, has implications for how chronic stress may accelerate aging and the onset of disease.

In the current study, the relationship between chronic psychological stress and the cellular damage that underlies aging and disease is explored here, replicating the prior elderly participant study with a sample of 49 young adults (ages 18-26) via direct salivary assay. The more non-invasive method of salivary assay shows high correlation with both blood serum and urinary measurements (Levine, et al. 2007). It was hypothesized that chronic psychological stress, as measured by the presence of high basal cortisol over time, would positively correlate with DNA damage as indications of accelerated aging also in younger populations.

Methods

Participants

Undergraduate students (n = 49) were recruited from a medium sized Mid-western university, ages 18-26 years (mean age = 19.4 years; SD = 1.66). All persons were enrolled in the general education course, Introduction to Psychology, and received partial course credit for

their voluntary participation. The participants were mostly female (n = 39), as is typical of the gender majority enrollment of the course.

Measures

Salivary Assays

All samples were assayed in duplicate in the Psychoneuroendocrinology Lab (PNEL) at the University of Northern Iowa using enzyme immunoassays. Research shows a strong correlation between salivary and serum levels of both cortisol, and DNA damage biomarkers making salivary assays representative of a minimally invasive and accurate technique for hormone/biomarker measurement (Salimetrics, 2016).

Procedure

Upon arrival, participants were informed that the current research was investigating how nutrition and various other social, personality and attitudinal factors were related to various biomarkers collected via a saliva sample. After providing informed consent consistent with the IRB approved protocol, participants were asked to provide an initial saliva sample using the passive drool procedure advocated by Salimetrics (2016). After providing an initial saliva sample, participants were seated at a computer terminal and instructed that they would be completing a series of questionnaires. A second saliva sample was collected from participants after approximately 1 hour, and a third sample at the end of the research session (approximately 1.5 hours). During the session, participants completed computer-based questionnaires related to personality traits and psychological distress as well as their nutritional intake over the previous 12 months, existing levels of social support, physical health symptoms, and experiences with social exclusion by others within their social realm (which are not part of the current research report). Participants provided the three samples over the approximate 90-minute time span in the

midday hours (defined as between 10:30am and 2:30pm) via salivary assay, a time typical of a decline in normal basal cortisol (See Fig. 2). Cortisol levels were averaged from these samples. No specific stressor was applied. Although providing saliva samples and/or completing online questionnaires may be considered mildly stressful for some, the participants self-select into the study based on a variety of research choices from the university research credit for participation system. No other recruitment methods were used or compensation given. Due to deviation from normal distribution, a log-transformation was used for the mean cortisol measurements of the three samples. DNA damage biomarkers were measured from the second sample.

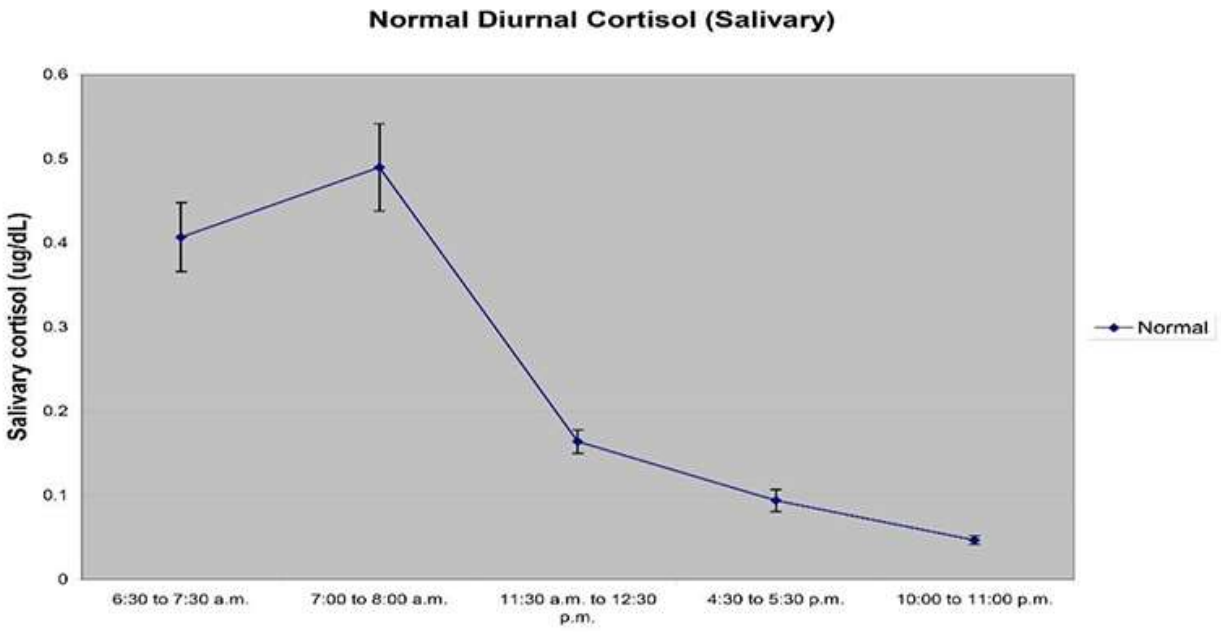

Fig. 2

Normal adult cortisol ranges and recommended time points for measurements in salivary assays (Salimetrics, 2016).

Results

The mean level of cortisol excretion was correlated with DNA oxidation, such that higher levels of cortisol were associated with higher levels of DNA damage at a significant level (Pearson's $r(47) = .35$, $p < .05$). The samples were taken from relatively healthy subjects with no known confounds of DNA damage, such as a history of cancer. Smokers were not controlled for due to the insignificant number of the sample ($n = 4$).

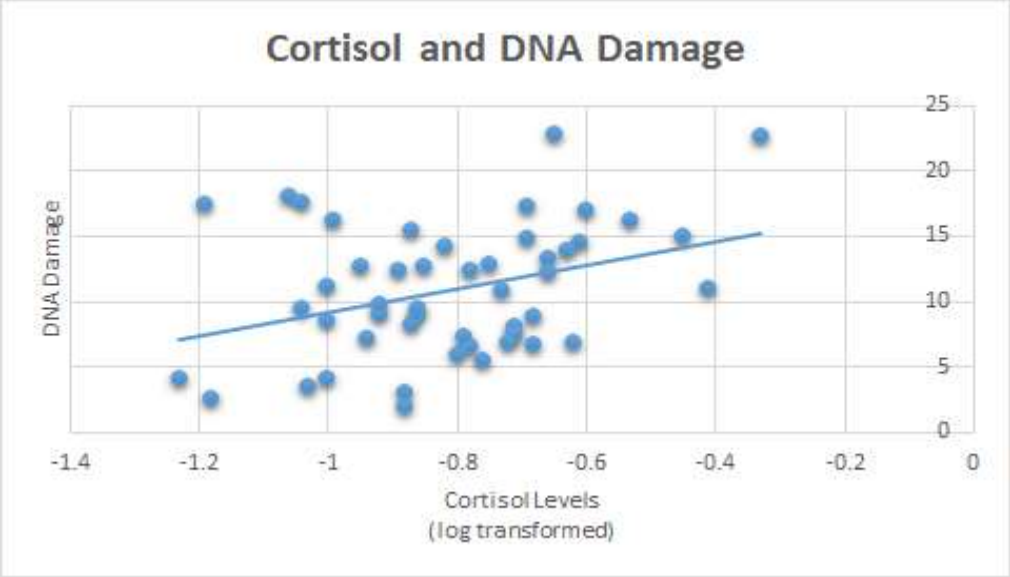

Fig. 3

Cortisol levels (log-transformed) correlate significantly with DNA damage.

Pearson's $r(47) = .35$, $p < .05$

Discussion

This is believed to be the first research to link elevated cortisol and oxidative DNA damage with a younger age group. Our findings build on prior research associating chronic

stress and accelerated aging with the elderly (Joergensen, et al.). Comparative research suggests a connection between youth chronic stress and cognitive decline in older ages (Sterlemenn, et al.). Thus, persistent elevated cortisol at younger ages may be implicated in initiating or accelerating negative health effects traditionally thought of as a function of aging itself. The association between aging and decreases in oxidative cell repair are well known (Hasan, et al. 2011), however the influence of stress hormones on these repair mechanisms are becoming clearer (Flint, et al. 2007). Given the disparity in the life expectancies between men and women, and research supporting sex differences in HPA axis reactivity (Wang, et al., 2007), this link may have a role in illuminating a biopsychological basis for sex differences in longevity across cultures.

The current study purports chronic stress as indicated by the presence of elevated cortisol during a time typical of a decline in cortisol levels and is limited to mean basal cortisol levels from the defined midday levels. This may restrict comparability to other methods of measuring chronic stress, such as cortisol deposited in hair samples (Stalder, et al., 2012) or serum or urine samples over more extended periods. Individual differences in diurnal patterns and levels of the cortisol awakening response were not accounted for. This may limit the generalizability of the results. It is believed, however, given strong correlation between serum and salivary cortisol (Salimetrics, 2016), that the current study represents an effective method for assessing chronic stress. Also, the sensitivity of salivary assays has shown clinical utility in physiological disorders of the HPA axis, such as Cushing's syndrome and adrenal insufficiency disease, showing strong correlations with serum cortisol at extreme ends of measurement (Raff, 2009).

Our research provides support for the link between elevated cortisol and oxidative DNA damage associated with aging and disease. Further research should include a larger more

representative sample that could be statistically controlled for sex differences, and different methods of measuring chronic stress as indicated by elevated cortisol. Because chronic stress is implicated in numerous negative health outcomes, an assessed chronic stress condition from a clinical perspective could be a predictor for many stress related illnesses, including depression, anxiety related disorders, posttraumatic stress disorder, heart disease, diabetes, and cancer (Qi & Rodrigues, 2007). Future research into the epigenetic changes associated with prolonged elevations of cortisol are warranted. The biological underpinnings along with the psychological impacts make the human stress response system a true biopsychological phenomenon that can be managed with strategies to improve maladaptive coping styles along with possible future medications to help regulate the HPA axis. If this connection between chronic stress and DNA damage throughout the lifespan is reinforced through future research, preventative interventions can be employed earlier for more positive impacts on health.

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